

THE PARASITISM OF *ALECTRA VOGELII* BENTH. WITH SPECIAL REFERENCE TO THE GERMINATION OF ITS SEEDS.

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INTRODUCTION.

Although the root-parasites are well represented among the South African flowering plants, comparatively little is known about their life-histories, anatomy and ecology. The only species which has been studied fairly well, is *Striga lutea* Lour. (Witchweed). This semi-parasite causes serious damage to gramineous crops and is therefore of considerable economic importance. From the work of Pearson (1911, 1912 and 1913), Stephens (1912 *a* and *b*) and Saunders (1933) it is clear that its life-history quite closely resembles the life-cycles of the European semi-parasites.

Besides the witchweed, a few other South African root-parasites are of economic importance. This paper deals with one of these, viz. *Alectra vogelii* Benth., a semi-parasite which, though not so prevalent as *S. lutea*, is nevertheless a serious drawback to the cultivation of leguminous crops in certain areas of the Transvaal and Rhodesia.

In Dyer's "Flora Capensis" (Vol. IV, Sect. 2) (1904) Hiern does not retain the genus *Alectra* Thunb. (Benth. et Hook. f. Gen. Pl. ii, 996), but refers it to *Melasma* Berg. (Benth. et Hook. f. Gen., Pl. ii, 996). In the "Flora of Tropical Africa" (Vol. IV, Sect. 2) (1906) Hemsley and Skan are, however, of the opinion that it is better to follow Bentham in retaining both genera. They evidently attach considerable diagnostic importance to the fact that in *Melasma* the calyx becomes inflated in fruit, and use it to distinguish between the two genera. They also state: "The species of *Melasma* are not any of them root-parasites as far as we can tell."

A. vogelii is evidently of tropical origin, occurring at present in regions well north of the equator (Northern Nigeria) from which it extends to regions as far south as the Cape Province (Upington). Most of the specimens of this species in the South African herbaria have been collected in Southern Rhodesia (in the vicinity of Salisbury) and in the Northern

Transvaal. In the Western Transvaal the parasite occurs in the districts of Lichtenburg and Schweizer-Reneke. Most of the material for this investigation was collected at the Sannieshof School Farm (Dist. Lichtenburg), where the infestation is rather heavy at present.

THE LIFE-CYCLE AND ECONOMIC IMPORTANCE OF *A. vogelii*.

The life-cycle of the parasite is completed within a period of three to four months. When cowpeas are planted in January or February in infested soil, the first parasite plants appear about six weeks later. During the subterranean period the young plant lives holo-parasitically. With the appearance of the growing point of the stem above the soil surface, the plant enters the semi-parasitic stage of its life-history. The aerial parts become green and the parasite is able to synthesise its own organic foods, taking only water and mineral foods from the host plant. A few weeks after the appearance of the parasite, the first flowers open and within a period of about a month or six weeks later the first capsules are ripe. The seeds, which are extraordinarily minute, are produced in great profusion. They are, furthermore, especially adapted to wind dispersal by having loose, funnel-shaped testae which render them light so that they can be carried long distances. With the increasing cultivation of its host plants (leguminous crops) it can therefore be expected that this parasite may in the future become much more widely distributed than it is at present.

With regard to the damage caused by the parasite, it may be stated that it resembles *S. lutea*. The attacked host plants show unmistakable signs of wilting, even when the water content of the soil is still relatively high. Under conditions of drought the attacked plants wilt before those which are free from the parasite. The injury done to the host thus consists mainly in that the latter is robbed of its water supply. This may take place to such an extent that the host pines away and eventually dies. Like many other semi-parasites among the Scrophulariaceae, *A. vogelii* is therefore primarily a "water parasite". As the parasite has a very reduced root system, which is totally devoid of root hairs, it is, however, clear that there can be no appreciable absorption of inorganic nutrients directly from the soil and that these substances also have to be obtained from the host.

Other symptoms of the injury caused by the parasite are : the attacked plants are markedly smaller than those not attacked, they have a yellowish colour and produce practically no seeds. On heavily infested soil the crop is consequently very poor and not profitable.

The age at which the damage caused by the parasite becomes noticeable

in cowpeas varies with individual plants of the same variety. In some cases the different developmental stages of the parasite and host plant take place more or less simultaneously, whereas in others the host plant has already been killed and shrivels up before the parasite begins to flower. These differences are probably due to variations in the resistance of individual host plants towards the parasite, although other factors as, for example, the number of parasites per host plant and variations in the environmental conditions, may also play an important part.

THE GERMINATION OF THE SEEDS.

The seeds of some root-parasites germinate under more or less the same conditions as those of non-parasitic plants. Heinricher (1917), for instance, has found that the seeds of *Euphrasia* and *Alectorolophus* germinate readily under the usual conditions of moisture and temperature. In the case of many other root-parasites (semi-parasites as well as holoparasites), however, germination takes place only under the influence of a chemical stimulus, which has its origin in the roots of the host plant. This phenomenon was observed *inter alia* by Heinricher (loc. cit.) with *Tozzia* and *Lathraea*, by Garman (1903) with *Orobancha minor*, by Kusano (1908) with *Aeginetia indica*, by Saunders (loc. cit.) and Brown and Edwards (1944) with *Striga lutea*, and by Chabrolin (1937-1938) with *Orobancha speciosa*.

As far as Saunders (loc. cit.) could determine, the substance which is necessary for the germination of the seeds of *S. lutea* is exuded by the roots of species belonging to the Gramineae only. Thus there seems to be a very close relationship between the parasite and its host plants in this case: only the host plants exude the active substance and all of them are members of the Gramineae.

According to Chabrolin (loc. cit.) no such specific host-parasite relationship exists in the case of *Orobancha speciosa*. The active substance necessary for the germination of the seeds of this parasite, is exuded by several species, some of which are not naturally related to each other. The capacity of the roots to activate the germination and their capacity to nourish the parasite are two independent properties which co-exist in the habitual hosts only.

When the present investigation was started, the first problem was to determine to which of the above categories the seeds of *A. vogelii* belong. Are the seeds able to germinate independently of the host plants, and, if not, does there exist a specific relationship between the parasite and its hosts as far as the germination is concerned?

1. *Preliminary Experiments*.—Several attempts were made to germinate the seeds in the absence of any host factor. In each case the seeds were sown between two pieces of filter paper, then placed on fine river sand in a Petri dish, and moistened with tap water. The cultures were usually incubated at 30°C and daily examined under the microscope. Although some cultures were kept under these conditions for a month and longer, no germination was observed.

In the next experiment the possibility that the germination may be host-dependent was tested. The technique of Saunders (loc. cit.) for the germination of seeds of *S. lutea* was modified to suit the purpose. Seven cowpea seeds were planted in fine river sand in an earthenware pot with a pinch-cock outlet at the bottom. When the seedlings were about two weeks old an over-abundance of water was added to the pot. After 24 hours the excess was drawn off, and the "filtrate" obtained in this way was used in the germination tests. The Petri dish culture method was again adopted. One lot of seeds of the parasite was kept moist with the "cowpea filtrate"—fresh "filtrate" being added daily—while a second lot of seeds, which served as a control, was kept moist with tap water. Incubation took place at 30°C. Two days after the first treatment, it was observed that some of the seeds which had been moistened with the "cowpea filtrate", were already germinating. After a week the percentage germination in this dish had reached 47·5, whereas not a single germinated seed was observed in the control dish.

This experiment was repeated several times and the results obtained were similar. It is therefore clear that the roots of the cowpea plants exude a substance or substances (hereafter referred to as "active substance"), the presence of which is essential for the germination of the seeds of *A. vogelii*. After this fact had been definitely established, the question arose as to whether the roots of other species, host plants as well as non-hosts, also exude the active substance. This problem was not studied immediately, because it was thought necessary first to improve and to standardise the experimental technique.

2. *The Influence of Previous Exposure to Moist and Warm Conditions on the Germination of the Seeds*.—Certain observations which were made when endeavouring to induce germination in the absence of the host factor led to a considerable improvement in the experimental technique. In one of these experiments the seeds were kept moist at 30°C for two weeks, at the end of which period they still showed no signs of germination. They were then transferred to a Petri dish containing dry sand, a fresh "cowpea filtrate" was added, and incubation at 30°C was resumed. Though no further additions of "filtrate" followed, it was observed that two days later most of the seeds were germinating and on the fourth

day the percentage germination had reached about 80. This percentage was much higher than the values obtained by daily adding fresh "cowpea filtrate" to the seeds, as described above. If the fact is borne in mind that a single treatment of the dry seeds with "cowpea filtrate" causes no germination at all, this result becomes the more striking. The most obvious explanation of it is that the previous exposure of the seeds to the moist and warm conditions made them more sensitive towards the action of the stimulating substance. The next series of experiments was designed with the object of testing this probability more critically.

The essential feature of these experiments was that different lots of seeds were exposed for varying periods of time to moist conditions at 30°C, before aliquot portions of a "cowpea filtrate" were added. The Petri dish culture method was applied and the following quantities were used: about 300 seeds placed upon 100 gm. of sand were moistened with 25 ml. of tap water. After the period of exposure had elapsed, the filter papers with the seeds were transferred to Petri dishes containing 100 gm. of dry sand each, 25 ml. of the filtrate was added in each case, and the cultures were again incubated at 30°C. The possible influence of differences in the concentration of the active substance was eliminated by choosing the times when the imbibition was started in such a way that the periods of previous exposure to warm and moist conditions (hereafter termed "pre-exposure") elapsed simultaneously. The lots of seeds used in each experiment could consequently all be treated simultaneously with aliquot portions of the same "filtrate". The results of the first experiment are set forth in Table 1.

TABLE 1.

The Effect of Length of Previous Exposure to Moisture at 30°C on the Percentage Germination. (First Experiment).

Time of Pre-exposure in hours.	Percentage germination.
0	0
12	1.3
24	15.6
36	31.1
48	30.4

These results indicate an increase in the percentage germination when the period of pre-exposure is lengthened. As the percentage germination was, however, only about 30 with a pre-exposure of 48 hours, there was reason to believe that this period was still too short to obtain the maxi-

num sensitivity of the seeds towards the active substance. In a second experiment, the results of which are recorded in Table 2, longer periods of pre-exposure were consequently applied.

TABLE 2.

The Effect of Length of Previous Exposure to Moist Conditions at 30°C on the Percentage Germination. (Second Experiment).

Time of Pre-exposure in days.	Percentage germination.
0	0
1	29·2
3	40·3
5	42·7
6	68·7
7	75·6
8	82·0
9	85·2
10	82·9

These results clearly suggest that the seeds are sensitised towards the active substance by pre-exposure. As the length of the pre-exposure increases, so does the percentage germination until an optimum exposure time is reached on about the eighth day. With pre-exposure periods of from 8 to 10 days the percentage germination remains more or less constant at a high level.

Another point of interest was what the effect of an unusual lengthening of the pre-exposure on the percentage germination would be. Under field conditions the seeds of the parasite are sometimes exposed for relatively long periods to a high soil moisture content and it is therefore of practical importance to know how such conditions affect the germination capacity.

To investigate this question an experiment was carried out in which the periods of pre-exposure varied from 14 to 40 days.

TABLE 3.

The Effect of Long Periods of Previous Exposure to Moisture at 30°C on the Percentage Germination.

Time of Pre-exposure in days.	Percentage germination.
14	98·6
20	94·6
30	86·0
40	84·3

The results, which are recorded in Table 3, indicate that when the seeds have once reached their maximum sensitivity towards the active substance, they evidently retain this sensitivity for at least another 30 days under moist and warm conditions.

The results of the last three experiments are in agreement with those obtained by Chabrolin (loc. cit.), but differ partly from those obtained by Brown and Edwards (loc. cit.). The former found that the seeds of *Orobanche speciosa* reached a maximum sensitivity towards the stimulating substance after having been pre-exposed to moist conditions for 15 days. After a month there were still no signs of secondary inhibition. Brown and Edwards (loc. cit.), working with the seeds of *Striga lutea*, also observed that pre-exposure enhanced the rate of germination. They, however, found that after the optimum time of pre-exposure had been reached, the germination capacity fell with a further prolongation of the period of pre-exposure. This difference between the results of Chabrolin and mine on the one hand, and those of Brown and Edwards on the other, may be due to differences among the root-parasites in this respect.

3. *The Technique applied in the Subsequent Germination Tests.*—After the fact had been established that exposure of the seeds to moist and warm conditions before the active substance is added, enhances the rate of germination, the procedure of pre-exposure was included in the further germination tests. The details of the method applied are the following: From 200 to 400 seeds are sown between two pieces of filter paper which are placed upon 100 gm. of fine sand in a Petri dish and moistened with tap water. The lid of the dish is replaced, and the culture is incubated for ten days at 30°C. During this period care is taken that the culture remains moist. The pieces of filter paper with the seeds are then transferred to another Petri dish containing 100 gm. of dry sand. 25 ml. of a "cowpea filtrate", which is obtained as already described, is immediately added. After another incubation period of 4 days at 30°C the percentage germination is determined with the aid of a binocular microscope.

As all the seeds cannot be brought into the field of the microscope simultaneously, they are counted as follows: the germinated seeds are first counted and removed with a double needle. Then a thin glass plate, ruled into small, numbered squares, is placed over the remaining seeds and the number in each square is determined. From these counts the percentage germination is calculated.

In general, good results were obtained with this technique. The germination was not only rapid, but also fairly uniform, as is indicated by the data presented in Table 4. These results were obtained by simul-

taneously doing twelve determinations on portions of the same seed sample. Aliquot portions of the same "cowpea filtrate" were used to supply the active substance in constant concentration. The value of the standard deviation calculated from these data is ± 3.2 .

TABLE 4.

Variation of the Percentage Germination as determined by the Method described in the Text.

No. of determination.	Percentage germination.
1	87.4
2	93.8
3	91.7
4	94.4
5	91.3
6	91.7
7	97.7
8	97.5
9	96.2
10	96.4
11	90.9
12	95.8

In the application of this technique during the rest of the investigation, certain modifications had sometimes to be introduced to suit the particular requirements of the experiments concerned.

4. *The Plant Species which exude the Active Substance.*—The next question to be investigated was whether the roots of plant species other than the cowpea also exude the active substance. The species which were tested included several leguminous plants as well as two non-leguminous species. In each case about seven seeds of the species to be tested were planted in an earthenware pot containing moist sand. When the plants were still young (from about two weeks to a month old) and growing vigorously a "filtrate" was obtained in the usual way. In some cases, where the seedlings were small and slender, for example *Sphenostylis angustifolia*, a culture solution was added to the sand in the pot. Only some weeks later, when the plants were growing healthily, the culture solution was drawn off and the sand in the pot was thoroughly rinsed with tap water, before an excess of water was added in order to prepare the "filtrate". In most cases, especially where the "filtrate" caused no germination, the tests were repeated several times. The results are presented in Table 5.

TABLE 5.

The Influence of "Filtrates" obtained from Cultures of Several Plant Species on the Germination of the Seeds of *A. vogelii*.

Name of Species.	Percentage germination.
Wild Cowpea (<i>Vigna sinensis</i>)	±80
Wild Vetch (<i>Vicia ervilla</i>)	±20
Spring Vetch (<i>Vicia sativa</i>)	± 5
Winter Vetch (<i>Vicia sativa</i>)	±10
Soybean (<i>Glycine hispida</i>)	Over 80
Ground-nut (<i>Arachis hypogaea</i>)	0
<i>Stizolobium deeringianum</i>	Over 80
<i>Dolichos donchos</i>	±80
Mung-bean (<i>Phaseolus aureus</i>)	Over 80
Sunnhemp (<i>Crotalaria juncea</i>)	0
Pigeon Pea (<i>Cajanus indicus</i>)	2
Sugar Bean	Over 80
Broad Bean (<i>Vicia faba</i>)	Over 80
Garden Pea (<i>Pisum sativum</i>)	0
<i>Sphenostylis angustifolia</i>	±10
Maize (<i>Zea mays</i>)	0
Sunflower (<i>Helianthus annuus</i>)	0

From these data it is clear that the "filtrates" obtained from the non-leguminous species (*Zea mays* and *Helianthus annuus*) did not cause germination, whereas nearly all the leguminous species activated the germination, the only exceptions being Sunnhemp, ground-nuts and the garden pea. The germination of the seeds of *Striga lutea* is activated by maize roots, and it is therefore clear that the substance necessary for the germination of the witchweed seeds is evidently not identical with that which activates the germination of the seeds of *A. vogelii*. It may be that the latter substance is exuded by the roots of leguminous species only, but more evidence is required on this point. The fact that the results were negative with three of the leguminous species, although the tests were repeated several times in each case, indicates that some members of the Papilionaceae apparently do not exude the active substance. It may, however, be that the roots of these plants exude the substance in such small quantities that it cannot be detected by the technique used in these experiments. That this may be the case is strongly suggested by the fact that I did not succeed in inducing germination with "filtrates" of ground-nuts, although some agriculturists assert that *A. vogelii* parasitises *Arachis hypogaea* in certain areas. The herbarium specimens of *Alectra* recorded as parasitising *Arachis hypogaea*, especially those of Galpin (8865 and 9046 both in Nat. Herb. Pretoria) and that of MacKay

(in Nat. Herb. Pretoria) were examined, and they were found to belong to *A. vogelii*. In view of these facts it was decided to attempt some other means of establishing whether or not the roots of ground-nuts are able to activate the germination of the seeds of *A. vogelii*. Several experiments were performed in which the roots of the ground-nut plants were brought into direct contact with the seeds of the parasite. This was done as follows: a piece of blotting paper was rolled cylindrically to fit the inside of a gas cylinder. The paper was moistened, and after the seeds were sown on its outer surface, it was pushed into the cylinder. A germinating seed of *Arachis hypogaea* was placed between the blotting paper and glass wall along the upper rim of the cylinder. The culture was kept moist by a small amount of water in the bottom of the cylinder. On the outside the cylinder was covered with dark paper and its mouth was plugged with cotton wool. The culture was kept at room temperature and was examined under the microscope at regular intervals.

As the taproot of the ground-nut seedling grew downwards, its branch-roots spread over the seeds of the parasite on the blotting paper, thereby coming into direct contact with many of them.

The results of the first experiments, which were carried out during the autumn under relatively cool conditions, proved to be negative. When they were repeated during the next summer, several of the seeds in contact with the host roots germinated. Although the percentage germination was low in all cases, it was high enough to exclude the possibility of independent germination (i.e., germination of a very low percentage of seeds of the parasite in the absence of the host factor) as exhibited by some parasites, e.g. *Striga lutea*. It was therefore concluded that the roots of ground-nuts also exude an active substance, though in very minute quantities. In fields infested with *A. vogelii*, where the roots of the ground-nut plants are able to come into direct contact with the seeds of the parasite, these small quantities of the exuded substance are evidently sufficient to cause a fair amount of germination.

Another point of interest in Table 5 is the fact that *Sphenostylis angustifolia* also caused germination of the seeds of the parasite. This leguminous species is indigenous to Southern Africa and occurs rather abundantly in certain localities in the vicinity of Potchefstroom. I have as yet not come across any evidence of *A. vogelii* occurring in the veld; in all cases that have come to my notice the parasite grew on cultivated leguminous crops. In a letter accompanying one of his specimens of *A. vogelii* to the Division of Botany and Plant Pathology, Pretoria, Galpin also wrote: "Though the neighbourhood was well collected over on many occasions, I never met with this species in the veld and do not know what its indigenous host plant is". As already mentioned, *A.*

vogelii is apparently of tropical origin. Some of its host plants, viz. certain cowpea varieties, are also indigenous to tropical Africa. The question now arises as to whether the parasite spread to the southern parts of Africa only after the cultivated host crops had been introduced into these parts, or whether the distribution of the parasite took place via uncultivated leguminous species which are indigenous to these southern parts. The fact that *Sphenostylis angustifolia* causes germination of the seeds of the parasite indicates that the second probability can not be dismissed, even though this species of *Alectra* has, to my knowledge, not yet been observed on uncultivated plants.

5. *The Influence of Various Concentrations of the Active Substance on the Germination.*—To determine the influence of various concentrations of the active substance on the germination, a "cowpea filtrate" obtained in the usual way, was used as initial solution. The concentration of the active substance in this "filtrate" was taken to be 100 arbitrary units. By diluting portions thereof with the appropriate amounts of tap water, solutions were obtained with concentrations of 5, 10, 25, and 50 arbitrary units. The activity of these solutions was tested in the usual way on sensitive seeds of the parasite.

The results, which are shown in Fig. 1, Curve A, clearly indicate that the concentration of the active substance has a pronounced effect on the percentage germination. With an increase in the concentration of the substance the percentage germination increases at a fairly rapid rate, until a concentration of about 25 per cent. of that of the mother solution used in this experiment is reached. With a further increase in the concentration the promoting effect becomes less pronounced. If it is taken into consideration that the total amount of substances exuded by the roots of the host plant is very small, and that the active substance probably constitutes only a small portion of this amount, it is clear that the concentration of this substance in the mother solution was very low. Nevertheless a solution having a concentration of only 5 per cent. of that of the mother solution caused germination of some of the seeds. It is therefore clear that the stimulating substance is biologically active even in extremely minute quantities.

A second experiment was carried out along similar lines, except that the active substance was applied in the form of an extract of cowpea roots. Five gm. of fresh cowpea root material with 100 ml. of distilled water was squashed in a mortar, and the mixture was filtered through dry cotton wool. The extract obtained in this way served as a mother solution, the concentration of which was again taken to be 100 arbitrary units. For the rest the procedure was the same as that of the previous experiment.

The results obtained agree quite closely with those of the last experiment, except that a more regular curve is obtained when they are represented graphically (Fig. 1, Curve B). From these data it is also clear that, if low concentrations of the active substance are applied, an increase in the concentration causes a greater increase in the germination percentage than when relatively high concentrations are applied. Furthermore, these results also indicate that the substance is active even in very low concentrations. When the sap of 5 gm. of root material was diluted with 2,000 ml. of water, the resulting solution still caused germination in about 16 per cent. of the seeds.

6. *The Influence of Temperature on the Active Substance.*—To get a better insight into the nature and physiological significance of the active substance, a study of its properties was undertaken. First of all its behaviour at various temperatures was investigated.

A fresh "cowpea filtrate" was obtained in the usual way and divided into aliquot portions. Each portion was quickly heated to the desired temperature, kept at this temperature for ten minutes and then cooled under running tap water. The influence of each of the following temperatures was studied: 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 97°C (boiling point). Germination tests were then carried out with these pre-treated solutions.

TABLE 6.

The Effect of Temperature on the Stability of the Active Substance.

Temperature to which heated (°C).	Percentage germination.
30	76.2
40	75.3
50	78.2
60	65.6
70	54.2
80	29.5
90	10.4
97	0

The data recorded in Table 6 clearly indicate that the active substance is thermo-labile. Temperatures of 50°C and lower have no observable effect on the substance, but its activity is diminished by exposure to 60°C and higher temperatures. The higher the temperature, the more quickly the process of inactivation takes place. At boiling point the substance was completely inactivated within ten minutes.

In its thermo-lability the active substance resembles an enzyme. According to the present views on the nature and constitution of enzymes,

their thermo-lability is due to the fact that they are either proteins or proteids. It is, of course, possible that the active substance may be, like enzymes, of a proteinaceous nature without necessarily being an enzyme. Its thermo-lability may also be due to other causes. The substance may, for instance, be volatile and may therefore be expelled from solution at high temperatures.

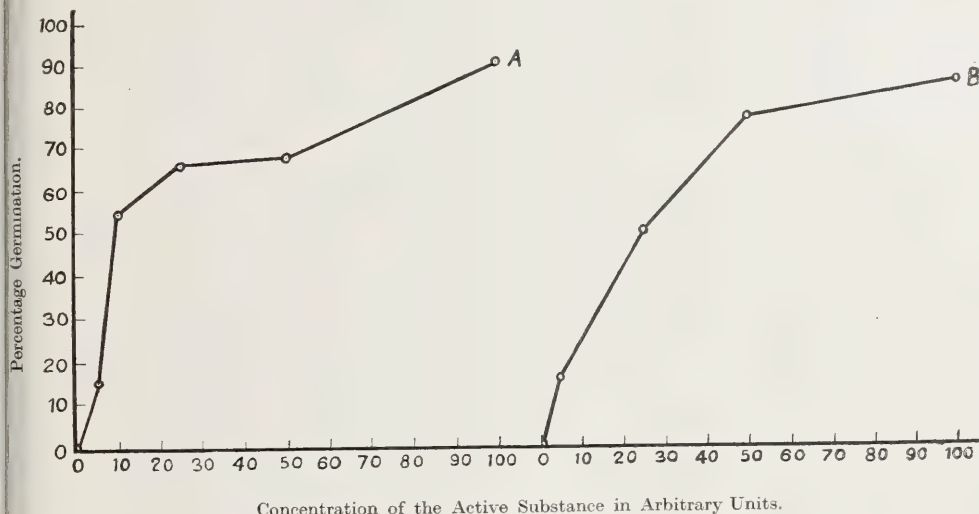


FIG 1.

The effect of different concentrations of the active substance on the germination of the seeds.

Curve A : seeds treated with "cowpea filtrate".

Curve B : seeds treated with cowpea root extract.

The instability of the substance at high temperatures is, in any case, a strong indication that it is not identical with the auxins (auxin *a* and *b*) which have been isolated from higher plants, for according to Went (1928), Boysen Jensen (1936) and others these auxins are heat-stable.

7. *The Effect of Ageing on the Stability of the Active Substance.*—

Having established the fact that the active substance is thermo-labile, the question arose as to whether it could retain its activity indefinitely at room temperature. Several experiments in this connection were carried out along the following lines : "Cowpea filtrates" containing the active substance were obtained in the usual way. 25 ml. of each "filtrate" was immediately added to sensitive seeds of the parasite. The rest of

the solution was kept in an unstoppered Erlenmeyer flask at room temperature. At varying time intervals portions of 25 ml. each were again removed to carry out germination tests. The seeds used in each test were taken from the same sample and had been previously sensitised in the usual way by incubation at 30°C under moist conditions for 10 days.

TABLE 7.

The Effect of Ageing on the Activity of "Cowpea Filtrates".
(*"Filtrates" stored at Room Temperature.*)

No. of Experiment.	Room Temperature (°C).	Age of "Filtrate" (Days).	Percentage Germination.
1	17—23	0	78.2
		2	34.9
		3	9.2
2	17—23	0	69.1
		2	3.6
		4	0
3	17—27	0	77.4
		2	35.5
		4	7.2
4	14—19	0	79.8
		2	87.7
		4	17.7
		7	1.4
5	13—19	0	78.4
		3	61.3
		7	3.0

From the results recorded in Table 7 it is clear that the active substance does not retain its activity when stored at room temperature. The rate at which the inactivation took place, was not the same in all the experiments. Whereas in some cases the "filtrate" was totally inactive after four days (Experiment 2), in other cases it still caused germination of some seeds after an ageing period of 7 days. (Experiments 4 and 5.) At low room temperatures the inactivation process evidently takes place at a slower rate than at higher temperatures. The length of the period in which the "filtrate" becomes totally inactive apparently also depends upon the initial concentration of the active substance.

The instability of the active substance at room temperature may be due to several causes, the most important being :

- (1) The substance may be volatile and may consequently escape from solution even at room temperature.

- (2) The substance may be oxidised by the oxygen of the air.
- (3) The substance may be sensitive to light and, as the "filtrates" used in the above experiments were exposed to daylight during the period of ageing, inactivation might have been caused by the light.
- (4) The substance may be decomposed by micro-organisms which may be present in the "filtrates".
- (5) The substance may undergo chemical changes such as decomposition, isomerisation, or denaturation. That such changes may occur in activation substances ("Wirkstoffe") obtained from plants, was demonstrated by several investigators. Kögl and his co-workers (Kögl, Koningsberger and Erxleben, 1936; and Koningsberger, 1936), for instance, have found that pure auxin becomes physiologically inactive within a few months even when sealed *in vacuo* and stored in the dark. The molecular weight remaining the same, this change is, according to them, due to isomerisation.

8. *Conclusion.*—The germination of the seeds of *A. vogelii* agrees in many respects with that of other root-parasites, e.g., *Striga lutea* and *Orobanche speciosa*. In general, the germination process can be divided into two phases, viz.: a first phase during which the seeds are sensitised under the influence of moist and warm conditions and a second phase which is initiated by the influence of an active substance exuded by the roots of the host plant and during which the primary rootlet starts growing.

Working on the seeds of *S. lutea*, Brown and Edwards (1944) describe the pre-exposure of the seeds to moist and warm conditions under the term "pretreatment." According to them the effects of pretreatment indicate that the host factor operates only at a comparatively late stage in the germination process. They maintain that certain developmental changes must occur in the seeds before the active substance can have any effect. These changes occur comparatively slowly, whereas those induced by the host factor are extremely rapid. They are, furthermore, of the opinion that pretreatment does not involve the acceleration of after-ripening, but simply promotes changes that belong to the germination process as such. In a later publication (1946) they put forward the tentative hypothesis that during pretreatment the seed itself forms a stimulating substance, which is the same as or similar to, that which originates in the host root.

The results obtained during the present investigation agree rather closely with those of Brown and Edwards, thereby indicating that in general there is great similarity between *A. vogelii* and *S. lutea* as far as

the germination is concerned. I feel inclined to agree with their conclusions in connection with the first phase of germination ("pretreatment"), but have to stress the fact that the present information bearing on this problem is still rather scanty. Before any definite conclusions can be arrived at our knowledge has to be supplemented by further investigation.

Furthermore, with regard to the nature of the active substance and its mode of action we still know very little. Perhaps some of the data on the germination of the seeds of the orchids with endotrophic mycorrhiza indicate the way for further investigation of this problem. Burgeff (1936) and afterwards his student, Schaffstein (1938), found that in the orchid genera, *Phalaenopsis* and *Euanthe*, the embryo suffers an avitaminosis, the mycorrhizal fungus supplying it with the missing factor necessary for germination. Furthermore, the living fungus can be replaced with an extract from it. Of still more importance is the fact that activation can also be effected by extracts of yeast or of wheat germ. These findings indicate that the relationship between the orchid and the symbiotic fungus is not as specific as was formerly believed. In the case of the angiospermous root-parasites with host-dependent germination, the relationship between the embryo of the parasite and the root of the host may be much the same. No definite conclusions can, however, be arrived at before more is known about the properties and physiological significance of the substances necessary for the germination of the seeds of various root-parasites. In a subsequent paper I hope to report on further investigation which is now being carried out in this direction.

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SUMMARY.

1. A short account is given of the life-history and economic importance of *A. vogelii*.

2. The roots of the host plants of the parasite exude a substance which is necessary for the germination of the seeds of the parasite.

3. Exposure of the seeds of the parasite to moist and warm conditions, before the active substance is added, enhances the rate of germination. When such pre-exposure forms part of the germination technique, fairly constant results are obtained.

4. Nearly all the leguminous species tested, exude an active substance, whereas the non-leguminous species did not activate the germination.

5. Although the stimulating substance is active even in very minute quantities, its concentration has, within certain limits, a pronounced effect on the germination.

6. The active substance is thermo-labile. When "cowpea filtrates" are exposed to temperatures of 60°C and higher they become less active. At boiling point such a filtrate is totally inactivated within 10 minutes.

7. When aged at room temperature in unstoppered flasks the active substance also becomes less active. Under these conditions total inactivation of a "cowpea filtrate" takes place within from 4 to 8 days.

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